High-Resolution Analysis of von Willebrand Factor Multimeric Composition Defines a New Variant of Type I von Willebrand Disease With Aberrant Structure but Presence of All Size Multimers (Type IC)

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In Type I von Willebrand disease, the whole series of von Willebrand factor (vWF) multimers is present in plasma, but all are decreased in quantity. No structural abnormality of individual multimers has been demonstrated so far in these patients. We now describe five individuals, from two unrelated families, who had this form of the disease and in whom the complex banding pattern of each vWF multimer was markedly abnormal. Inheritance was autosomal dominant and the clinical expression was mild. A bleeding history was elicited in three of the patients and included recurrent epistaxis, menometrorrhagia, and bleeding following tooth extraction. Replacement therapy had never been required. Although vWF levels in plasma were within the normal range in all of them, the ristocetin cofactor activity was decreased in four, and the bleeding time was prolonged in three. Analysis of vWF multimeric structure by agarose gel electrophoresis, including a newly developed high-resolution technique, demonstrated that the main band of each multimer was present, but a second, well-defined band always seen in normal individuals was missing in the patients. Two additional bands had altered mobility and were less well defined than in normal subjects, and a fifth, less intense band was also undetectable in the patients. Treatment with 1-deamino-8-arginine vasopressin (DDAVP) was assessed in two patients. It caused the circulating levels of vWF to increase and correct the bleeding time, but did not alter the structural abnormality. This study describes, therefore, a new variant form of Type I von Willebrand disease with aberrant structure of individual repeating multimers and an associated functional abnormality of vWF. In keeping with previously accepted terminology, the designation of Type IC von Willebrand disease has been adopted for this new variant.

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stacking gel was poured, 2.5 cm long, consisting of 0.8% high-gelling temperature agarose (FMC). Ten sample wells, each 10 mm × 2 mm, were cut 1 cm from the stacking/resolving gel interface, and 20-μL samples were applied. Electrophoresis was performed horizontally, at a constant current of 0.41 mA/cm and constant temperature of 16 °C, in a LKB Multiphor apparatus (LKB, Pleasant Hill, Calif) until the tracking dye was 0.5 cm from the anodal wick (usually, 18 hours). Long gels were 9 cm wide by 20 cm long by 1.5 mm thick and were cast and run as described for short gels, except that only four samples could be applied on each gel, and that electrophoresis was performed at 0.75 mA/cm. Identification of vWF multimers using 125I-labeled affinity-purified anti-vWF IgG was performed as described. All patient and normal control samples were stored in aliquots at −70 °C for comparable periods of time and routinely thawed only once immediately before testing. Repeated freezing and thawing, however, caused no appreciable change in the vWF multimeric pattern seen in these patients or in normal subjects. Scanning of selected autoradiographs was performed using a soft laser densitometer (Zeineh Model S1-504-XL, Biomed Instruments, Inc, Fullerton, Calif).

1-Deamino-8-D-arginine vasopressin (DDAVP; trade name: Stimate, Armour Pharmaceutical, Tarrytown, NY) was infused into two patients at a dose of 0.4 μg/kg following a protocol previously described. These studies were performed in Bari, Italy, and conducted in accordance with local rules regulating human experimentation after obtaining the informed consent of the patients.

RESULTS

Two unrelated families are described in this report and their pedigrees are shown in Fig 1. In family 1, the propositus was a 22-year-old Caucasian woman. She had never experienced abnormal bleeding, except for prolonged hemorrhage following extraction of a deciduous tooth in her childhood and extraction of a permanent tooth one year prior to this study. In both instances, bleeding was controlled by local hemostatic measures. She had one uncomplicated pregnancy and full-term delivery without bleeding complications. Her apparently normal son is now aged 3 years and has never shown an abnormal bleeding tendency. The propositus’ father died prior to these studies and had no history of a bleeding disorder. The propositus’ mother had a history of menometrorrhagia dating from her adolescence. She had two full-term pregnancies, and the deliveries were without bleeding complications. The propositus’ sister, ages 20 years at the time of this study, had a negative bleeding history. No other relevant anamnestic data could be elicited in this family.

In family 2, the propositus was a 19-year-old Caucasian woman who had experienced recurrent episodes of epistaxis since childhood. Her menses were prolonged, and the blood loss was increased. The propositus’ father, who is now aged 40 years, as well as her brother, who is now aged 15 years, also reported recurrent episodes of epistaxis. The propositus’ mother, on the other hand, had a negative bleeding history. No other relevant anamnestic data could be elicited in this family.

The results of pertinent parameters of hemostatic function are reported in Table 1. The propositus in family 1 had a prolonged bleeding time on all six occasions on which she was tested. Her mother had a bleeding time that varied between normal and slightly prolonged, whereas her sister had a prolonged value but was tested only once. In these three patients, there was a disproportion between the plasma level of ristocetin cofactor activity, which was low, and that of vWF antigen, which was normal or borderline. Factor VIII:C activity was modestly decreased in two and low/normal in one. All these measurements were normal in the propositus’ son, whose bleeding time was also normal. In family 2, the propositus had normal bleeding time on one occasion, but prolonged values were measured on four additional occasions. Bleeding time measurements were normal in both her mother and father, on two separate occasions each, but no value was obtained for her brother. Ristocetin cofactor activity was decreased only in the propositus and, as in family 1, the value was disproportionally low in relation to the level of vWF antigen, which was low/normal. Only a small aliquot of plasma from the brother of the propositus (II-2) was available and was used for analyzing the multimeric structure of vWF. The patient was not available for further testing.

Multimeric analysis of plasma vWF revealed structural abnormalities in three members of family 1 and three members of family 2 (Figs 2 and 3). On short gels, the full complement of multimers seen in normal subjects, including the largest one, was present in the patients, and the relative concentration of different size multimers was also similar to that of normal subjects. This finding was better shown by scanning the autoradiographs with a soft laser densitometer and comparing samples with equivalent levels of vWF antigen (Fig 4). Individual multimers, however, showed an abnormal banding pattern in all the patients here reported. Rather than the previously described triplet pattern seen in normal plasma (Fig 2, lower panel), only one band could be resolved in each repeating multimer. It was not as sharply defined as the predominant band in each normal triplet and its mobility was slightly increased as compared with the normal predominant band. Satellite bands were not evident in the affected members of family 1 even after prolonged exposure of the autoradiograph (Fig 2, upper panel). In the patients from family 2, however, some diffuse material reacting with the anti-vWF antibody was visible around the central band (Fig 3). The marked decrease of satellite bands could also be appreciated on the densitometric tracing of autoradiographs of short gels (Fig 4). Comparison with type I A von Willebrand disease samples indicated that this struc-
Table 1. Results of Relevant Laboratory Tests in the Patients Studied

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>Bleeding Time (min)</th>
<th>Platelet Count (x 10³/μL)</th>
<th>RIPA* (mg/mL)</th>
<th>VIII:C (U/dL)</th>
<th>vWF:Ag (U/dL)</th>
<th>RiCof (U/dL)</th>
<th>Structural vWF Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I-2</td>
<td>6–12</td>
<td>180</td>
<td>1.30</td>
<td>43</td>
<td>50</td>
<td>38</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>II-1</td>
<td>10–30</td>
<td>180</td>
<td>1.10</td>
<td>60</td>
<td>55</td>
<td>23</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>II-2</td>
<td>16</td>
<td>120</td>
<td>1.30</td>
<td>43</td>
<td>65</td>
<td>38</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>III-1</td>
<td>6</td>
<td>220</td>
<td>1.29</td>
<td>60</td>
<td>100</td>
<td>120</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>I-1</td>
<td>4</td>
<td>290</td>
<td>1.30</td>
<td>72</td>
<td>100</td>
<td>76</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>I-2</td>
<td>6</td>
<td>230</td>
<td>1.00</td>
<td>192</td>
<td>150</td>
<td>175</td>
<td>No</td>
</tr>
<tr>
<td></td>
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<td>6–15</td>
<td>195</td>
<td>1.28</td>
<td>60</td>
<td>55</td>
<td>26</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Normal subjects
- Mean: 4.16, 213, 0.91, 99, 96, 90
- Range: 2.31–6.01, 138–316, 0.72–1.10, 57–150, 49–145, 51–140

*RIPA (ristocetin-induced platelet aggregation) was measured by adding varying amounts of ristocetin to platelet-rich plasma and determining the concentration (expressed in milligrams per milliliter) that induced aggregation with an initial velocity corresponding to 30% of maximum (see ref. 9).

When more than one value is given for the bleeding time, these values represent the range observed. The results of VIII:C, vWF:Ag, and ristocetin cofactor (RiCof) activity are expressed in arbitrary units (U) per deciliter, where 100 U is the amount present in 1 dL of pooled normal plasma. Results in normal subjects represent either arithmetic mean ± SD (bleeding time, platelet count, RIPA) or geometric mean with 95% confidence limits (measurement of VIII:C, vWF:Ag, and RiCof).

tural abnormality was not the consequence of the decreased plasma levels of vWF (Fig 4). This finding, however, was better shown using the long gel technique described below.

Improved resolution of the complex multimeric structure of vWF was achieved with the use of longer gels of higher resolving power. A repeating pattern could be identified in normal plasma vWF analyzed under these conditions (Figs 5 and 6). Each multimer, identified by a bracket, consisted of a central predominant band (indicated as number 1) and four satellite bands, two moving slower (1A and 2A) and two moving faster (1B and 2B) than the central band. The latter appeared as a closely spaced doublet, a finding that was better evident in the fastest moving multimer identified (first from the bottom; Fig 5). Some additional bands were also seen with a mobility faster than that of the first multimer and may represent the product of proteolysis. All these findings were consistent in more than 20 different normal samples analyzed. In three affected members from family 1 (Fig 5)
patients was not simply due to decreased plasma levels of vWF (Fig 7).

Fig 4. Soft laser densitometer scanning of a short gel. The top sample is that of an apparently normal individual who had a vWF plasma level of 44%; the middle panel (IC vWD) represents the sample from patient I–2 with vWF plasma level of 60%; the bottom panel (IA vWD) shows that of a patient with type IA von Willebrand disease who had a vWF plasma level of 22%. Note that multimers of all sizes are present in the sample from the newly described patient and that the concentration of smaller multimers relative to the largest is very similar in all samples. The structural alteration shown in Figs 2 and 3, with decrease of satellite bands, can be appreciated here as lack of the inflections preceding and following the main peak corresponding to the predominant band in each multimer. These inflections can be detected in the smaller multimers, both in normal and IA vWD.

and in two affected members from family 2 (Fig 6) studied with the high-resolution technique, plasma vWF showed abnormalities of the complex multimeric structure. Band 1 was detectable in all multimers whose structure could be resolved, but band 1B was always missing in the patients. Band 1A was markedly decreased (Figs 5 and 6), although it could sometimes be detected, particularly in the fastest moving multimer. Bands 2A and 2B were also much less evident than in normal, were not as sharply resolved, and showed a slightly faster mobility than the normal counterparts (Figs 5 and 6). Scanning of the autoradiographs made the structural abnormality of individual multimers well evident. Comparison with samples containing similar or lesser amounts of vWF antigen demonstrated that absence or marked decrease of certain bands in these newly described patients was not simply due to decreased plasma levels of vWF (Fig 7).

One patient from family 1 (II-1) and one from family 2 (II-1) were treated with DDAVP, and plasma samples obtained at various times after the infusion were analyzed. Both patients responded with a three- to fourfold increase in the circulating levels of vWF antigen. Multimeric analysis of vWF on short gels demonstrated increased intensity of the bands that were already present before the infusion of DDAVP, and also the appearance of a new, discrete band that was particularly evident in the smaller multimers (Fig 8). On longer gels of greater resolving power, the complex vWF multimeric structure remained clearly abnormal after the infusion of DDAVP (Fig 9). Band 1B in all multimers was still missing, in spite of increased amounts of circulating vWF, but band 1A became slightly more evident, although always with much less intensity than in normal samples. Band 2A increased slightly in intensity, whereas band 2B showed a very marked increase in concentration. This latter finding was not evident in the fastest moving multimer (first from the bottom), although it was very apparent in the two...
The two families described here were characterized by a mild congenital hemorrhagic disorder transmitted as an autosomal dominant trait. Spontaneous bleeding, when present, was of mucosal type and limited to epistaxis and menometrorrhagia. No patient had ever been subjected to a major surgical challenge, but bleeding after tooth extraction in the propositus of family 1 could be stopped by local hemostatic measures. The bleeding time was always prolonged in two of the patients, but it was borderline or normal on some occasions in the others. Ristocetin cofactor activity was decreased in four of five individuals tested, although their plasma vWF concentration was normal or borderline low. Because of these findings, and of the structural abnormality of vWF identified, the patients were diagnosed as having von Willebrand disease.

The vWF molecule has a complex multimeric structure and the pattern characteristic of normal individuals is now well defined. Two distinct examples of aberrant banding patterns have been reported so far in patients with von Willebrand disease, namely type IIC and type IID variants. In type IIA and IIB variants, the relative concentration of bands in each multimer is different from normal, but no abnormality in the electrophoretic mobility of individual bands has been observed. Common to all these variants is the lack of the larger vWF multimers in plasma. The patients described here, in contrast, are characterized by the presence of all vWF multimers in plasma, with normal relative concentrations of different size multimers. Because of this, they fall into the category that has been designated type I von Willebrand disease. At variance with the previously reported types IA and IB, however, these patients show aberrant structure of each individual multimer and therefore represent a new subgroup. The abnormality seen in the multimeric structure of their vWF is unique. Although it could be appreciated, in part, upon analysis of the more commonly used short agarose gels, such abnormality became more evident upon analysis of the long gels. Band 2B as seen on long gels, with its increased intensity, is likely to correspond to the new band seen on short gels after DDAVP treatment (compare Figs 8 and 9). The pattern seen after infusion of DDAVP was identical in the two patients from the two different families. The bleeding time shortened, 30 minutes after the infusion, from ten minutes to four minutes in the patient from family 1, and from seven minutes and 30 seconds to five minutes in the patient from family 2. There was no change in the platelet count after treatment.
Fig 8. “Short gel” sodium dodecyl sulfate agarose electrophoresis of samples obtained before and at various times after infusion of 1-deamino-8-D-arginine vasopressin (DDAVP). Patients are identified as shown in Fig 1. The arrows indicate bands that are more evident after DDAVP infusion. Refer to Fig 2 for additional details.

Fig 9. “Long gel” sodium dodecyl sulfate agarose electrophoresis of samples obtained before and at various times after infusion of 1-deamino-8-D-arginine vasopressin (DDAVP). Refer to Fig 5 for details. Note that band 1B is missing in the patient’s samples even after infusion of DDAVP and consequent rise in the levels of circulating vWF. Note also that following the infusion of DDAVP there is increased intensity of band 2B in the multimers above the first from the bottom (arrows).

more evident with the use of longer gels of higher resolving power. A marked derangement in the complex banding pattern seen in each repeating vWF multimer was apparent in six patients from the two different families, and the abnormal pattern was consistent in all of them.

In previous studies, it has been determined that the fastest moving multimer of vWF has a mobility corresponding to the dimer of the constituent subunit. Therefore, the series of bands here designated as I in each repeating multimer is likely to represent the association of an increasing number of such dimers. The intervening bands seen with the high-resolution agarose gel electrophoresis may result from post-translational changes of the multimers initially synthesized in endothelial cells, giving rise to oligomers of varying mol wt within each multimer. It is likely, therefore, that the abnormal pattern of intervening bands seen in these patients is the reflection of primary structural defects resulting in the generation of modified oligomers different from normal. Aberrant structure of individual vWF multimers has been described so far only in type II von Willebrand disease, in association with lack of the largest multimers. The patients described here, however, present a unique structural abnormality of vWF and yet show the presence of all multimeric forms in their plasma. Therefore, they provide evidence that an altered molecular structure of vWF may also be present in patients with type I von Willebrand disease.

The structurally abnormal vWF molecule synthesized in these patients appears to be functionally impaired. Although plasma vWF concentrations were within the normal range, or only borderline low, levels of ristocetin cofactor activity were comparatively decreased in the affected individuals in spite of the presence of all multimeric forms. In patient I-1 from family 2, however, the levels of plasma vWF were higher than in all other patients. His bleeding time and ristocetin cofactor activity were in the normal range even though the structural vWF abnormality was identical to that of the other patients. Such an observation suggested that correction of the hemostatic derangement could be achieved by increasing the circulating levels of the patients' own endogenous vWF. This was in fact demonstrated by the infusion of DDAVP in two patients with lower levels of vWF and prolonged bleeding time. In both cases, the bleeding time normalized in parallel with the posttreatment increase in plasma vWF concentration and in spite of the persistence of the vWF abnormality. This latter finding provides additional
evidence that the marked decrease of some satellite bands in individual vWF multimers cannot be the simple consequence of decreased plasma levels in the patients, because the abnormality persisted when such levels were well within or above the normal range following DDAVP infusion.

In conclusion, this study describes a new variant of von Willebrand disease characterized by a distinctive structural alteration of the vWF molecule but with the presence of the full complement of multimeric forms in plasma, including the largest. Nevertheless, the molecule is less functional than normal and patients exhibit a bleeding diathesis even with circulating levels of vWF antigen that are only at the low limit of normal. This study also demonstrates that correction of the bleeding time abnormality can be achieved with the infusion of DDAVP. This drug, therefore, represents the first choice for treating hemorrhagic episodes in patients such as those described here. In keeping with previously adopted terminology, we propose the designation of type IC von Willebrand disease for this newly defined variant form.

REFERENCES

High-resolution analysis of von Willebrand factor multimeric composition defines a new variant of type I von Willebrand disease with aberrant structure but presence of all size multimers (type IC)

G Ciavarella, N Ciavarella, S Antoncicchi, D De Mattia, P Ranieri, J Dent, TS Zimmerman and ZM Ruggeri